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(54) Title: 1,5-ANHYDROHEXITOL NUCLEOSIDE ANALOGUES AND PHARMACEUTICAL USE THEREOF (57) Abstract <p>It has been found that 1,5-anhydrohexitol nucleoside analogues, wherein a 4-substituted-2,3,4-trideoxy-1,5-anhydrohexitol is coupled via its 2-position to the heterocyclic ring of a pyrimidine or purine base, exhibit remarkable anti-viral properties against herpes viruses, pox viruses and related viruses. The new nucleoside analogues are represented by general formula (I) wherein B is a heterocyclic ring derived from a pyrimidine or purine base, X represents H, N₃, F, Cl, Br, I, amino, -NHR², -N(R²)₂, -OR², -SR² or CN, R¹ and R² are the same or different and hydrogen, alkyl, acyl or phosphate groups are represented, or wherein X is hydrogen and a double bond is situated between the 3- and 4- position of the 1,5-anhydrohexitol ring. Pharmaceutically acceptable salts and esters thereof are included.</p>		

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**1,5-ANHYDROHEXITOL NUCLEOSIDE ANALOGUES AND
PHARMACEUTICAL USE THEREOF**

Technical field

This invention relates to nucleoside analogues with an aglycone six-membered ring which exhibits remarkable antiviral activities. This invention further relates to the chemical synthesis and the pharmaceutical and/or medical use of such nucleoside analogues.

Background

Pentofuranosyl nucleosides are nucleosides in which a pentofuranose ring, that is, a heterocyclic five-membered ring, which is derived from pentose sugars, is bonded to the heterocyclic ring of a pyrimidine or purine base. Substituents can be present on each of both rings. Ring atoms as well as pendant hydroxy and amino groups can be replaced by other atoms or groups whereby a large number of possible variations is created.

Different pentofuranosyl nucleosides are known for their anti-viral activities. Nucleosides for example with a 2-deoxy-2-fluor-D-arabinofuranose moiety have a potential anti-viral activity against herpes viruses and are among the most active anti-herpes agents. Compare De Clercq et al., Biochem. Pharmacol. 33, 2159 (1984). A number of these nucleosides has already been tested in vivo. Their antiviral activity is dependent on the presence of a virus-specific thymidine kinase, whereby they are converted into the corresponding 5'-monophosphate derivatives. The monophosphates are further phosphorylated by cellular enzymes to triphosphates which then inhibit the viral DNA polymerase.

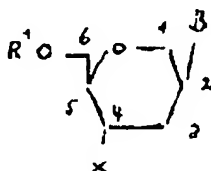
In the same manner base modifications of the natural 2'-deoxy nucleosides can provide these nucleotides with an anti-viral activity against herpes viruses. This activity of for instance 5-iodo-2'-deoxyuridine and E-5-(2-bromovinyl)-2'-deoxyuridine is likewise dependent on a virus-specific

thymidine kinase. Compare De Clercq et al., in Developments in Anti-viral Chemotherapy, pages 21-42 (1980), Ed. Collier and Oxford, Acad. Press.

Description of the invention

5 The present invention relates to 1,5-anhydrohexitol nucleoside analogues, wherein a 4-substituted-2,3,4-tri-deoxy-1,5-anhydrohexitol is coupled via its 2-position to the heterocyclic ring of a pyrimidine or purine base. They are represented by the formula I:

10



(I)

15

wherein B is a heterocyclic ring which is derived from a pyrimidine or purine base, and

wherein X represents a hydrogen atom, azido, F, Cl, Br, I, amino, $-NHR^2$, $-N(R^2)_2$, $-OR^2$, $-SR^2$ or CN,

20 wherein R^1 and R^2 are the same or different and represent hydrogen, alkyl, acyl or phosphate groups; wherein

30 alkyl is a straight or branched chain, saturated or unsaturated, substituted or non-substituted hydrocarbon radical with 1-20 carbon atoms; and acyl is an alkanoyl or aroyl group, wherein alkanoyl is an alkylcarbonyl radical and wherein alkyl is as described above and aroyl is a benzoyl, substituted benzoyl or naphthoyl;

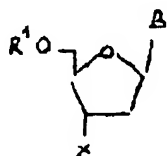
or wherein X is hydrogen and a double bond is situated between the 3- and 4- position of the 1,5-anhydrohexitol ring.

Pharmaceutically acceptable salts and esters of the compound of formula I are included in the invention.

The nucleoside analogues of formula I are new compounds. They display a certain similarity with 2'-deoxy-2'-phosphoribanosyl nucleosides of formula II wherein B, R^1 and X

have the same designation as in formula I, except for the enlargement of the ring with a methylene group between the ring oxide and the carbon which is coupled to the base.

5



(II)

According to the invention it has been found that the nucleoside analogues of formula I and their salts and esters exhibit remarkable anti-viral properties against herpes viruses, pox viruses and related viruses. Different analogues are selectively inhibiting for Herpes simplex virus type 1, Herpes simplex virus type 2, Varicella zoster virus and Cytomegalo virus. A new class of anti-herpes agents has therefore been found.

A number of nucleoside analogues has already been described by ourselves and others, which analogues contain a pyranose group (as well as pentoses and hexoses), but not a single one has been described as possessing anti-viral activities. Compare Herdewijn et al., Nucleosides, Nucleotides 10, 119-127 (1991) (pentoses, 2-deoxy-2-fluoropentopyranoses, inactive); Herdewijn et al., Bull. Soc. Chim. Belg. 99 895-901 (1990) (hexoses, inactive); Kaluza et al., Acta Chem. Scand. 44 294-296 (1990) and Hansen et al., Liebigs Ann. Chem. 1079-1082 (1990) (3-azidopyranoid analogues of AZT, inactive); Nord et al., J. Med. Chem. 30, 1044-1054 (1987) (2-deoxy-hexopyranoses, from inactive to very low activity). Until now it has not been found of a single hexose nucleoside that it is a substrate for cellular or viral kinases and thereby has an anti-viral effect. Insertion of an additional oxygen or nitrogen in the pentofuranose group, whereby analogues were created with a dioxane or morpholine moiety, equally did not provide the obtained compounds with any desired anti-viral properties. Compare Van Aerschot et al., Bull. Soc. Chim. Belg. 99 769-777 (1990).

The fact that anti-viral activities are found among the nucleoside analogues of formula I must be deemed surprising despite their configurational analogy with nucleosides of formula II. The effect of enlarging the pentofuranosyl ring to a 1,5-anhydrohexitol ring could not be anticipated beforehand. This is illustrated by the absence of anti-viral properties in the above mentioned derivatives.

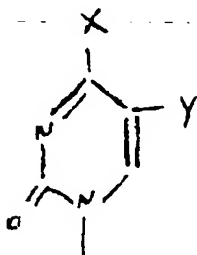
The invention also relates to pharmaceutical compositions from the nucleoside analogues of formula I and, where possible, to the use of these nucleoside analogues in therapy, for instance in the treatment or prophylaxis of virus infections, in particular herpes virus infections, for example herpes simplex virus types 1 and 2, Cytomegalo virus and Variella Zoster virus.

More detailed description of the invention
Compounds

The invention will now be described in more detail. The compounds according to the invention are nucleoside analogues wherein a 4-substituted-2,3,4-trideoxy-1,5-anhydrohexitol is coupled via its 2-position to the heterocyclic ring of a pyrimidine or purine base. They can be represented by the above stated formula I, wherein B, R' and X have the above stated designations. Pharmaceutically acceptable salts and esters are likewise included.

The hexitol has the (D)-configuration and the base and the X substituent have the (S)-configuration.

Group B is derived from a pyrimidine or purine base. When derived from a pyrimidine base it can be represented by formula III:



(III)

wherein X represents OH, NH₂ or NHQ,
 Q is OH or C₁₋₅ alkyl,
 Y is H, F, Cl, Br, C₁₋₅ alkyl, haloethyl or CH=CH-R wherein R
 represents halogen or C₁₋₅ alkyl and haloethyl with 1-4 F, Cl
 5 or Br atoms.

When B is a heterocyclic ring which is derived from a
 purine base it can be an adenine, guanine, hypoxanthine or
 xanthine ring, optionally substituted by halogen, C₁₋₅ alkyl
 or -CH=CH-R, wherein R represents hydrogen, halogen or C₁₋₅
 10 alkyl.

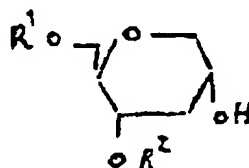
In addition, aza, deaza, deoxy or deamino analogues of
 each of the said heterocyclic rings, optionally with one or
 more of above mentioned substituents, can be present in the
 compounds of formula I.

15 Substituents R¹ and X have the designation as stated
 above.

Chemical synthesis

The nucleoside analogues of the present invention can
 be prepared in different ways. In a preferred method the
 20 corresponding (R¹, R²) substituted 1,5-anhydrohexitol ring
 protected in appropriate manner is first produced with a
 hydroxyl residue in its 2-position in the (R) configuration
 (formula IV).

25



(IV)

30

Activation with a leaving group provides nucleophile
 replacement with a purine or pyrimidine base, followed by
 deprotection of the desired nucleoside analogues (formula
 XIII). Substituents in 4-position (position X in formula I)
 35 can be introduced in accordance with classical and known
 reaction schedules which are used for introduction of
 substituents X in formula II (2'-deoxypentofuranosyl
 nucleoside analogues).

In similar manner the preparation of the 1,5-anhydrohexitol ring can be performed in different ways. A preferred method is elucidated in the following schedule.

The synthesis begins with glucose (V) which is converted into tetra-O-acetyl-glucopyranosyl bromide (VI) in accordance with Kartha et al., J. Carbohydrate Chem. 9, 777-781 (1990).

Reduction is achieved with tri-n-butyltinhydride [which can be generated in situ from dibutyltin oxide and a polymethylhydrosiloxane, in accordance with Kocienski et al., Carbohydrate Res. 110, 330-332 (1982)], or with other reducing means which provide compound VII. Removal of the acetyl groups with sodium methoxide is followed by introduction of a benzylidene protective group, analogously of protection of methylglucoside [Methods in Carbohydrate Chemistry, vol. 2, p. 208] whereby compound VIII is obtained. Selective reaction of the hydroxyl in position 2 is feasible after previous activation with dibutyltin oxide. Position 2 can either be selectively protected, for instance as an ester (for example $R = CH_3C_6H_4CO$) or can be functionalized with a leaving group (for example $R = CH_3C_6H_4SO_2$, formula IX). The hydroxyl group in position 3 is subsequently removed [(for instance by Barton deoxygenation, see Barton et al., Tetrahedron Lett. 30, 2619-2622 (1989))] whereby the compound of formula X is obtained.

Coupling of the purine or pyrimidine base can be performed substantially in three ways:

a) by nucleophile replacement of the leaving group in position 2 with the respective purine or pyrimidine base. Compare for example Medich et al., Tetrahedron Lett. 28, 4131-4134 (1987).

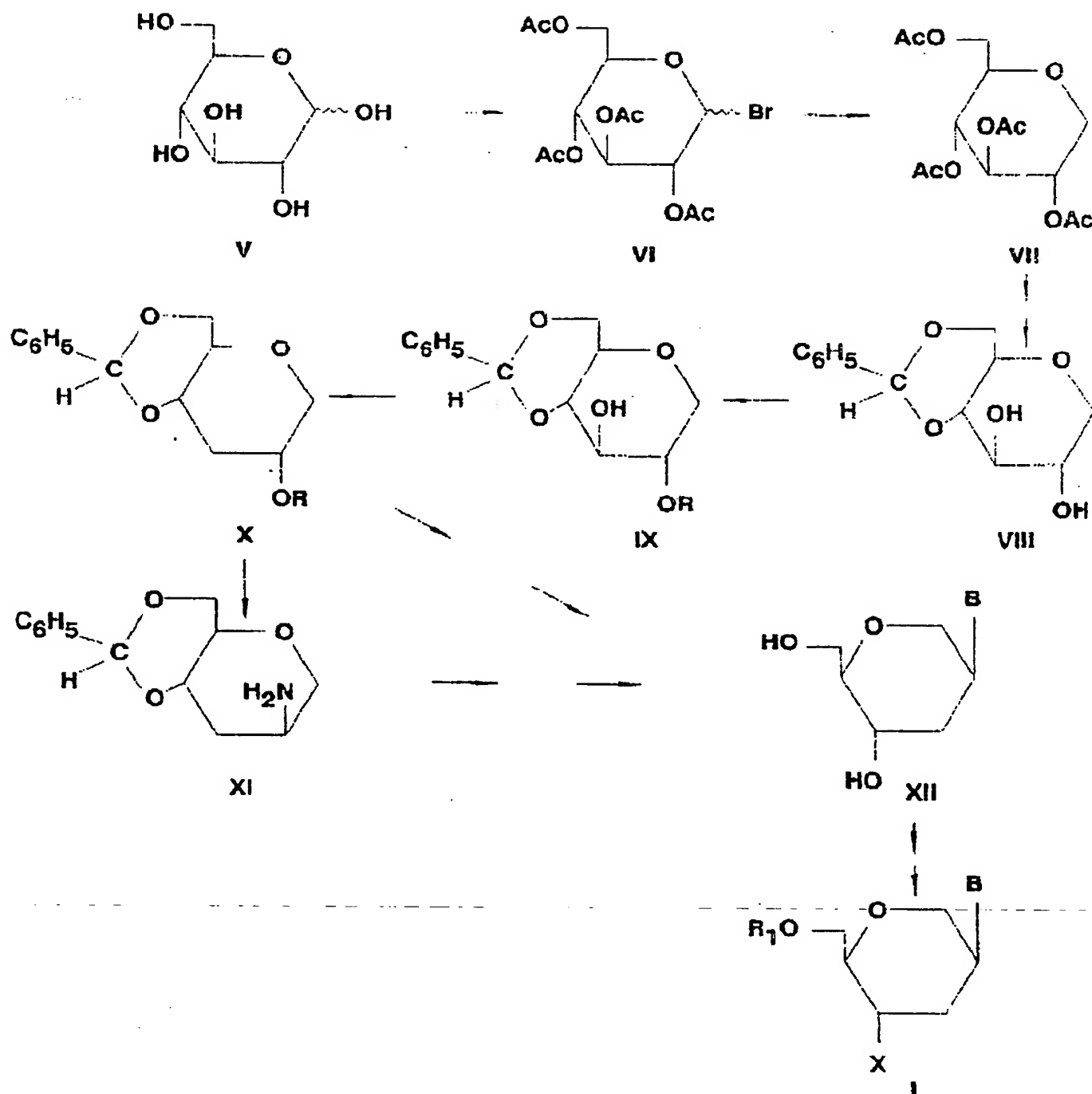
b) by hydrolysis of the temporary protective group R, whereby the compound of formula X is obtained, wherein $R = H$, followed by alkylizing of the purine or pyrimidine base under modified Mitsunobu conditions. Compare Jenny et al., Tetrahedron Lett. 32, 7029-7032 (1991).

c) by constructing the heterocyclic base by standard procedures after introduction of an amine function in the

(S) configuration (formula XI). For a survey of the construction of the base for a carbocyclic amine compare Marquez and Lim, Medicinal Res. Rev. 6, 1-40 (1986).

The resulting product of formula I can be purified by 5 standard procedures. In the alternative case a hydroxyl group in the 3-position can be removed during reduction after introduction of the base in the 2-position.

Pharmaceutically acceptable salts and esters of the nucleoside analogues of formula I can further be prepared in 10 conventional manner.



As stated above, the nucleoside analogues of the present invention generally have anti-viral activities against herpes viruses, pox viruses and related viruses, such as herpes simplex virus 1, herpes simplex type 2, 5 varicella zoster virus, cytomegalo virus and vaccinia virus. In this manner they can advantageously be used for treating the diseases caused by such viruses in human and veterinary medicine.

Pharmaceutical compositions

10 Pharmaceutical compositions containing the nucleoside analogues of the invention as an active ingredient can take the form of tablets, capsules, powders, suspensions, solutions, emulsions as well as salves and creams, and can be used for parenteral (intravenous, intradermal, 15 intramuscular, intrathecal etc.) injections, oral, rectal, intravaginal and intranasal administering or for local application (for instance on skin injuries, mucosa and eyes). Such compositions can be prepared by combining the active ingredient(s) with pharmaceutically acceptable 20 excipients normally used for this purpose. Such excipients can comprise aqueous and non-aqueous solvents, stabilizers, suspension agents, dispersing agents, moisturizers and the like, and will be known to the skilled person in the pharmaceutical field. The composition may further contain 25 likewise suitable additives such as for instance polyethylene glycoles and, if necessary, colorants, fragrances and the like.

The pharmaceutical compositions will preferably contain at least 0.1 volume % by weight of the active ingredient. 30 The actual concentration will depend on the disease and the chosen administering route. In general this concentration will lie between 0.1 and 100% for the above applications and indications. The dose of the active ingredient to be administered can further vary between 0.1 mg and 100 mg per 35 kg body weight, preferably between 0.1 mg and 50 mg per kg body weight, and most preferably between 0.5 mg and 20 mg per kg body weight.

The desired dose is preferably presented in the form of two, three, four, five, six or more sub-doses which are administered at appropriate intervals per day. These sub-doses can be administered in the form of dosage units containing for instance from 1 to 1500 mg, preferably from 5 to 1000 mg and most preferably from 10 to 700 mg active constituent per dosage unit, and if the condition of the patient permits the dose can, by way of alternative, be administered as a continuous infusion.

10 Examples

The compounds according to the invention as well as their chemical synthesis and the preparation of the starting materials are further illustrated in the following examples, which are not however intended to limit the invention.

EXAMPLES**5 2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosylbromide (1)**

This compound was prepared in accordance with Kartha et al., and Jennings, H., J. Carbohydr. Chem. 9, 777-781 (1990).

10 2,3,4,6-Tetra-O-acetyl-1,5-anhydro-D-glucitol (2)

To a solution of 44.8 g of compound 1 (109 mmol) in dry diethylether was added 55 ml bistributyltin oxide (109 mmol) and an equal quantity of polymethylhydrosiloxane (55 ml). The mixture was stirred at room temperature under nitrogen.

15 TLC evaluation after 3 hours (CH_2Cl_2 - MeOH 98:2) showed that all the 2,3,4,6-Tetra-O-acetyl- α -D-O-glucopyranosylbromide was converted into a more polar product. A solution of 15.80 g KF (2.5 eq, 272 mmol) in water was then added and the mixture stirred vigorously for

20 15 minutes. The Bu_3SnF precipitate was filtered and washed with diethylether. After separation of the water the ether layer was dried above anhydrous Na_2SO_4 and evaporated dry. The compound of the title (2) (30.06 g, 90.5 mmol; 83% yield) was obtained after chromatographic purification [1)

25 CH_2Cl_2 hexane 50:50; 2) CH_2Cl_2].

1,5 Anhydro-4,6-O-benzylidene-D-glucitol (3)

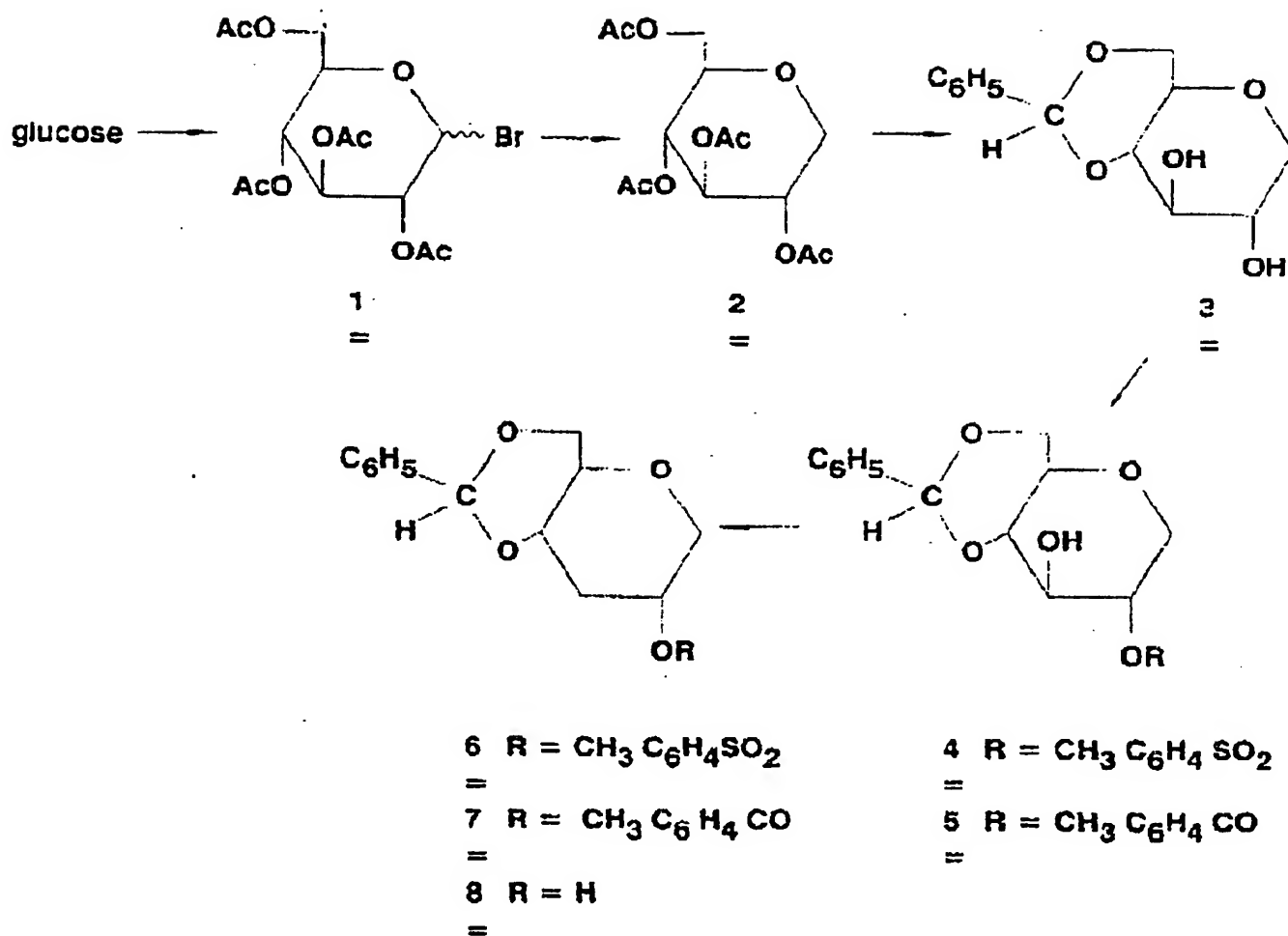
Removal of the protective groups of 2 was achieved by treating 30.06 g (90.5 mmol) of compound 2 with 400 ml 0.1 N

30 NaOMe for 2 hours at room temperature. The mixture was neutralized with acetic acid and evaporated dry. After CO evaporation with toluene, 12.4 g (91 mmol) freshly dried ZnCl_2 and 46.5 ml (455 mmol) benzaldehyde were added and the suspension stirred vigorously for 1 to 2 days at room

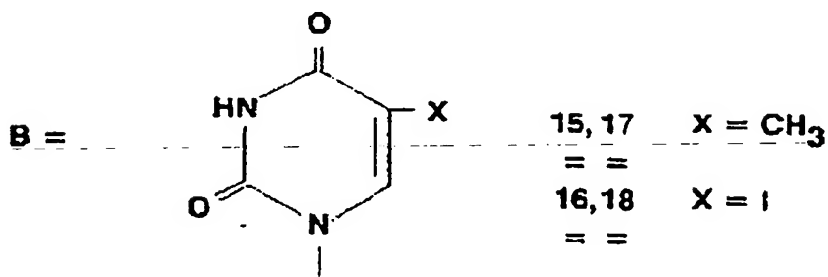
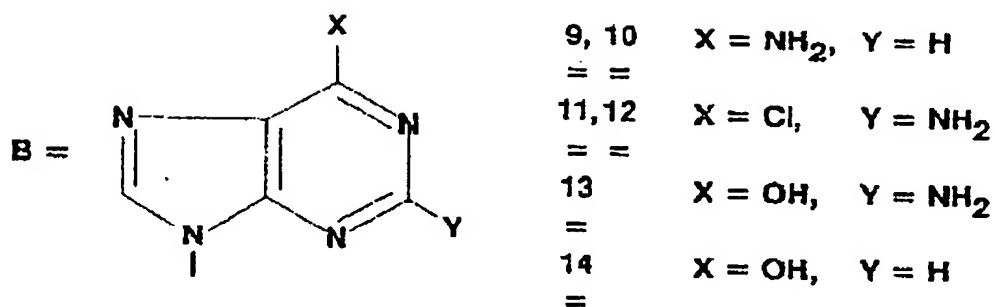
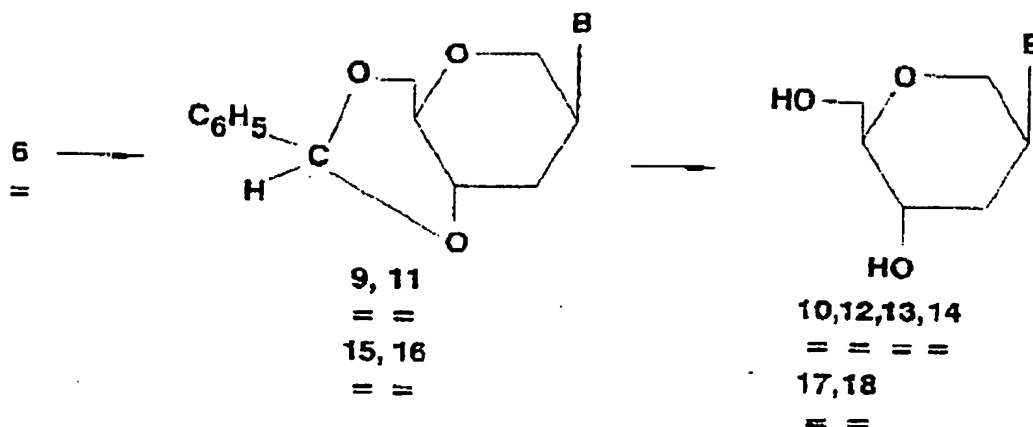
35 temperature.

The mixture was poured into cold water and extracted three times with ethyl acetate. The combined organic layer was dried on anhydrous Na_2SO_4 . After filtration and removal

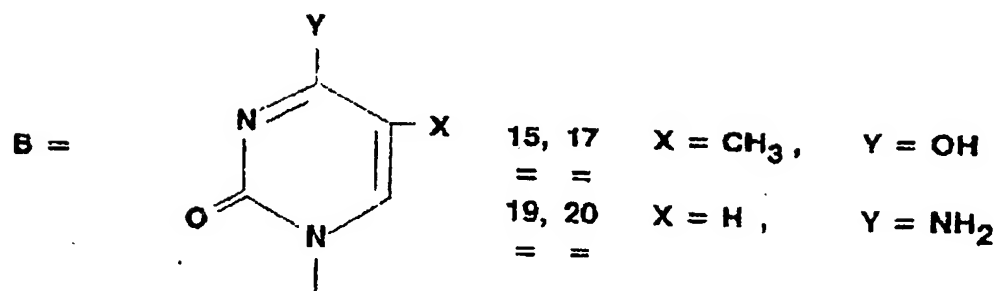
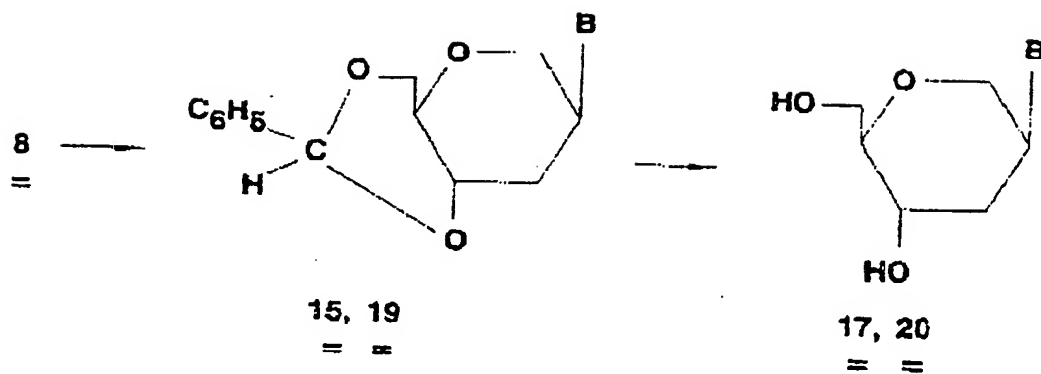
of the solvent the excess benzaldehyde was partially removed under vacuum at 70°C (oil pump). The solid residue was further purified by washing on a glass funnel with n-hexane followed by chromatographic purification [1) hexane - CH_2Cl_2 1:1; 2) CH_2Cl_2 ; 3) CH_2Cl_2 - MeOH 98:2] whereby 17.1 g (68 mmol) 75% yield) of compound 3 was obtained.



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1,5-Anhydro-4,6-O-benzylidene-2-O-p-toluenesulphonyl-D-glucitol (4)

The glucitol derivative 3 (8.5 g, 33.67 mmol) and dibutyltin oxide (8.38 g, 367 mmol) were suspended in 250 ml benzene. The mixture was heated under reflux for 16 hours with azeotropic removal of water. After removal of the volatile substances the residue was dissolved in 150 ml anhydrous dioxane and 7.06 g (37.04 mmol) p-toluenesulphonylchloride was added. The mixture was heated to 50°C for 6 hours, which resulted in a quantitative conversion to a less polar product. The mixture was concentrated, adsorbed on celite and purified by column chromatography (CH₂Cl₂ - hexane, 1:1; CH₂Cl₂;) to a yield of 11.22 g (27.6 mmol, 82%) of compound 4 as a white powder.

15

EIMS m/e : 406 (M⁺)

400 MHz ¹H NMR (DMSO-d₆) δ 2.42 (s, 3H, CH₃), 3.35-3.42 (m, H-4, H-5), 3.49 (t, J=11Hz, 1H, H-1α), 3.61 (m, 1H, H-6), 3.67 (m, 1H, H-3), 3.87 (dd, J=5.5Hz and 11Hz, 1H, H-1β), 4.14-4.25 (m, 2H, H-2, H-6'), 5.05 (s, 1H, PhCH), 5.12 (d, J=5.5Hz, 1H, OH), 7.35-7.50 (m, 7H, arom-H), 7.85 (m, 2H, arom-H) ppm.

90MHz ¹³C NMR (DMSO-d₆) δ 21.0 (CH₃), 66.9, 67.6 (C-1, C-6), 70.7, 70.8 (C-3, C-5), 79.2, 80.4 (C-2, C-4), 100.7 (PhCH) + 25 arom.

1,5-Anhydro-4,6-O-benzylidene-2-O-p-toluoyl-D-glucitol (5)

A suspension of the sugar derivative 3 (8.5 g, 33.67 mmol) and dibutyltin oxide (8.38 g, 33.67 mmol) in 250 ml benzene was boiled under reflux for 16 hours with azeotropic removal of water. The solution was concentrated and 150 ml dry dioxane was added. p-Toluoyl chloride (4.44 ml, 33.67 mmol) was added in droplets and the mixture was stirred for 5 hours at room temperature. The mixture was concentrated, adsorbed on celite and purified by column chromatography to a yield of 9.73 g (26.30 mmol, 78%) of compound 5 as a white powder.

1,5-Anhydro-4,6-O-benzylidene-3-deoxy-2-O-p-toluenesul-
phonyl-D-ribohexitol (6)

A) 11.22 g (27.6 mmol) of the tosylated sugar 4 and 23.60 g (193 mmol) of 4-dimethylaminopyridine (DMAP) were dissolved in 400 ml dry CH_2Cl_2 . The reaction mixture was cooled to -40°C and during vigorous stirring 2.53 ml thiophosgene (33.12 mmol) was added. The mixture was brought to room temperature. After stirring for 1 hour 6.30 g (38.64 mmol) 2,4-dichlorophenol was added and stirring continued for 2 hours. The mixture was poured into 300 ml 1 M KH_2PO_4 and extracted twice with CH_2Cl_2 . The organic layers were dried (Na_2SO_4), the volatile substances removed under vacuum and the residue purified by flash chromatography (hexane/ CH_2Cl_2 8:2 to CH_2Cl_2)

B) the obtained thiocarbonyl compound was dissolved in 300 ml anhydrous toluene. After fast boiling the solution for 10 minutes with N_2 , 7.84 ml (29.15 mmol) tri-n-butyltinhydride and 325 mg (2 mmol) 2,2'-azobis(2-methylpropionitrile) were added and the reaction mixture heated overnight at 80°C .

The mixture was evaporated and purified on silica gel with a yield of 6.90 g (17.67 mmol, 64%) of compound 6.
CMIS (NH_3) m/e : 391 (MH^+)

1,5-Anhydro-4,6-O-benzylidene-3-deoxy-2-O-p-toluenyl-D-
ribohexitol (7)

The reaction was performed as described for the synthesis of compound 6. Treating of 9.73 g (26.30 mmol) of the toluoylated hexitol 5 provided 6.79 g (19.73, 75%) of compound 7 after chromatographic purification.

1,5-Anhydro-4,6-O-benzylidene-3-deoxy-D-glucitol (8)

Removal of the toluoyl group of compound 7 was achieved by treating 6.79 g (19.73 mmol) thereof with 300 ml 0.1 M NaOMe for 4 hours at room temperature. After neutralizing and evaporation of the volatile substances the residue was purified by column chromatography (CH_2Cl_2 - MeOH, 99:1) with a yield of 3.72 g (15.81 mmol, 80%) of the above compound.

1,5-Anhydro-4,6-O-benzylidene-2-(adenin-9-yl)-2,3-dideoxy-D-arabinohexitol (9)

A mixture of 1.35 g (10 mmol) adenine, 400 mg sodium hydride (60% dispersion, 10 mmol) and 529 mg (2 mmol) 18-crown-6 in 60 ml dry DMF was stirred for 1 hour at 80°C. After adding a solution of 1.95 g (5 mmol) of compound 6 in 30 ml anhydrous DMF the stirring was continued for 16 hours at 100°C. The reaction mixture was cooled and evaporated dry. the residue was dissolved in ethylacetate (100 ml) and the organic phase was washed with saturated NaHCO₃ solution (50 ml) and H₂O (2 x 25 ml), dried and evaporated dry. The solid residue was purified by column chromatography (CH₂Cl₂ - MeOH, 97:3) with a yield of 989 mg (2.8 mmol, 56% yield) of compound 9. A quantity of 190 mg (0.49 mmol, 9%) of the tosylate 6, which had not reacted, was recovered.

UV (MeOH) : λ_{\max} 262 nm (ϵ = 11300)

MS (m/e) : 353 (M⁺)

¹H NMR (CDCl₃ + DMSO-d₆) δ 2.0-2.6 (m, H-3', H-3''), 3.5-4.55 (m, 5H), 4.94 (m, 1H), 5.57 (s, PhCH), 7.10 (br, NH₂), 7.35 (m, 5H, Ph), 8.19 (s), 8.27 (s) (H-2, H-8)ppm.

¹³C NMR (CDCl₃ + DMSO-d₆; internal ref. TMS) δ 32.6 (C-3'), 50.4 (C-2'), 68.3, 69.1 (C-1', C-6'), 73.6, 74.0 (C-4', C-5'), 101.2 (PhCH); 119.0 (C-5), 126.1, 127.8, 128.6, 137.6 (Ph), 139.0 (C-8), 149.5 (C-4), 152.5 (C-2), 156.1 (C-6)ppm.

1,5-Anhydro-2-(adenin-9-yl) 2,3-dideoxy-D-arabinohexitol (10)

The benzylidene moiety of compound 9 was hydrolyzed by heating 989 mg (2.8 mmol) thereof in 100 ml 80% acetic acid at 80°C for 3 hours. After evaporation and co-evaporation with toluene the residue was dissolved in water and washed with diethylether. The water layer was evaporated and the residue crystallized from MeOH-Et₂O with a yield of 602 mg (2.27 mmol, 81% yield) of compound 10.

mp : 237-239°C

UV (MeOH) : λ_{max} 261 nm ($\epsilon = 13500$)

CIMS (NH_3) m/e : 266 (MH^+), 136 (BH_2^+)

^1H NMR ($\text{DMSO}-d_6$) δ 1.7-2.4 (m, H-3', H-3'), 3.2-4.3 (m, 6H), 4.53-5.02 (m, H-5', 4'-OH, 6'-OH), 7.25 (br s, NH_2) 8.16 (s), 8.31 (s) (H-2, H-8) ppm.

^{13}C NMR ($\text{DMSO}-d_6$) δ 36.0 (C-3'), 50.2 (C-2'), 60.6, 60.9 (C-4', C-6'), 68.1 (C-1'), 83.1 (C-5'), 118.2 (C-5), 139.7 (C-8), 149.4 (C-4), 152.5 (C-2), 156.1 (C-6) ppm.

Anal.

10

1,5-Anhydro-4,6-O-benzylidene-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-arabinohexitol (11)

The 1,5-anhydrohexitol 6 (1.56 g, 4 mmol) and 848 mg (5 mmol) 2-amino-6-chloropurine were dissolved in 30 ml anhydrous DMF to which 830 mg (6 mmol) anhydrous potassium carbonate and 530 mg (2 mmol) 18-crown-6 were added. The mixture was stirred for 5 hours at 120°C after which the volatile substances were removed under vacuum and the residue adsorbed on silica gel. Purifying produced 295 mg (0.76 mmol, 90%) of the compound 11.

^1H NMR (CDCl_3) δ 1.86-2.32 (m, H-3') 2.45-2.75 (m, H-3''), 3.5-3.9 (m, 3H), 4.07 (dd, $J=2.6\text{Hz}$ and 13Hz , 1H), 4.34 (m, 2H), 4.77 (m, 1H), 5.30 (s, NH_2), 5.48 (s, PhCH), 7.2-7.5 (m, Ph), 8.27 (s, H-8) ppm.

^{13}C NMR (CDCl_3) δ 32.8 (C-3'), 50.8 (C-2'), 68.8, 69.2 (C-6', C-1'), 73.7, 74.6 (C-4', C-5'), 101.9 (PhCH), 125.9, 128.1, 128.9, 137.0, (Ph), 126.1 (C-5), 141.1 (C-8), 151.5 (C-6), 153.5 (C-4), 159.0 (C-2) ppm.

30

1,5-Anhydro-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-arabinohexitol (12)

The obtained compound 11 (295 mg, 0.76 mmol) was heated in 50 ml 80% acetic acid at 80°C to complete hydrolysis of the benzylidene moiety. Evaporation and co-evaporation with toluene 1 ft behind an oil which was purified on silica gel (CH_2Cl_2 - MeOH, 9:1). The product which precipitated after

concentration of the eluate provided 145 mg (0.48 mmol, 63%) of compound 12.

UV (MeOH) : λ_{max} 224 (27000), 249 (6100), 310 (8000) nm.
5 ^1H NMR (DMSO- d_6) δ 1.7-2.5 (H-3', H-3''), 3.94 (J=11Hz,),
4.18 (J=12Hz), 4.67 (t, J=5.5Hz, 6'-OH), 4.95 (d, J=5.2Hz,
4'-OH), 6.95 (s, NH₂), 8.30 (s, H-8) ppm.
 ^{13}C NMR (DMSO- d_6) δ 35.7 (C-3'), 50.3 (C-2'), 60.5, 60.7 (C-
4', C-6'), 67.8 (C-1'), 83.0 (C-5'), 123.0 (C-5), 141.9 (C-
10 8), 149.5 (C-6), 154.0 (C-4), 159.8 (C-2) ppm.

1,5-Anhydro-2-(guanine-9-yl)-2,3-dideoxy-D-arabinohexitol
(13)

A mixture of 145 mg (0.48 mmol) of compound 12 and 0.5
15 ml of a suspension of adenosine deaminase in 100 ml 0.05 M
phosphate buffer, pH 7.5, was incubated for 4 hours at 30°C.
The reaction mixture was concentrated to about 15 ml and the
precipitate filtered off. Recrystallization from water
provided 50 mg analytically pure compound 13. The filtrates
20 were placed onto an XAD column (25 x 2 cm), which was eluted
with water followed by MeOH-water (3:1). Evaporation gave an
extra 70 mg of compound 13 as a white product to a total of
0.43 mmol (89%).

smp

25 UV (MeOH)

CIMS (C_4H_{10}) m/e : (282 (MH⁺))

^1H NMR (DMSO- d_6) δ 4.52 (br, 6'-OH), 4.9 (br, 4'-OH), 6.56
(br, NH₂), 7.87 (s, H-8) ppm.

^{13}C NMR (DMSO- d_6) δ 36.3 (C-3'), 50.2 (C-2'), 61.0, 61.2 (C-
30 4', C-6'), 68.4 (C-1'), 83.2 (C-5'), 116.3 (C-5), 136.9 (C-
8), 151.5 (C-4), 154.1 (C-2), 157.9 (C-6) ppm.

Anal. ($\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_4$)

Calculated: C, 46.97; H, 5.38; N, 24.90

35 Found: C, 46.73; H, 5.40; N, 24.58

1,5-Anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-arabino-
hexitol (18)

A mixture of 2.60 g (10 mmol) of the sodium salt of 5-iodouracil [prepared in accordance with Colla L. et al., Eur. J. Med. Chem., 17, 569 (1982)], 1.95 g (5 mmol) crude tosylate 6 and 528 mg (2 mmol) 18-crown-6 in 80 mg dry DMF was stirred at 100°C for 16 hours. The reaction mixture was cooled and evaporated dry. The residue was dissolved in 100 ml EtOAc and the organic layer was washed successively with saturated NaHCO₃ solution (50 ml) and water (3 x 50 ml), dried and evaporated dry. Column chromatography (CH₂Cl₂ - MeOH, 98:2) produced 958 mg (2.1 mmol, 42%) yield of compound 16 in the form of an oil and 371 mg (0.95 mmol) of the tosylate, which had not reacted, was recovered.

The obtained oil was heated in 100 ml 80% acetic acid at 80°C to complete hydrolysis of the benzylidene moiety. The mixture was evaporated and co-evaporated with toluene and purified by column chromatography (CH₂Cl₂ - MeOH, 90:10) with a yield of 408 mg (1.11 mmol, 53%) of the compound 18 which crystallized out of MeOH.

mp 219-220°C

UV (MeOH) : λ_{\max} 282 nm

CIMS (NH₃) m/e : 369 (MH⁺)

¹H NMR (DMSO-d₆) δ 1.53-2.42 (m, H-3, H-3'), 2.8-4.2 (m, 6H), 4.53 (m, 1H), 8.47 (s, H-6) ppm.

¹³C NMR (DMSO-d₆) δ 35.3 (C-3'), 51.4 (C-2'), 60.7, 61.1 (C-4', C-6'), 67.2, (C-1'), 68.3 (C-5), 82.7 (C-5'), 147.9 (C-6), 150.9 (C-2), 160.9 (C-4) ppm.

Anal. (C₁₀H₁₃N₂O₅I x 0.75 H₂O) :

Calculated: C, 31.47; H, 3.83; N, 7.34

Found: C, 31.83; H, 4.14; N, 7.03

1,5-Anhydro-2,3-dideoxy-2-(thymine-1-yl)-D-arabinohexitol
(17)

The above compound was synthesized in the same manner from compound 6 but in very moderate

21/A
(corresponds with page 22
of Dutch record copy)

**NOT TAKEN INTO CONSIDERATION
FOR THE PURPOSES
OF INTERNATIONAL PROCESSING**

1,5-Anhydro-2-(cytosin-1-yl)-2,3-dideoxy-D-arabinohexitol
35 (20)

A suspension of 2.15 g (10 mmol) of N³-benzoylcytosine [prepared in accordance with Brown et al., J. Chem. Soc. 2384 (1956)], 1.18 g (5 mmol) of the alcohol 8 and 3.28 g

(12.5 mmol) of triphenylphosphine in 100 ml anhydrous dioxan was treated with 1.97 ml (12.5 mmol) diethylazodicarboxylate in 20 ml anhydrous THF for 15 hours at room temperature. After removal of the volatile substances the residue was resuspended in 100 ml EtOAc and washed twice with 50 ml water.

The organic layer was dried on anhydrous Na_2SO_4 , evaporated and adsorbed on silica gel. Purifying by column chromatography produced 800 mg (1.85 mmol, 37%) of the crude 1,5-anhydro-4,6-O-benzylidene-2,3-dideoxy-2-(N⁶-benzoylcytosin-1-yl)-D-arabinohexitol.

The benzoyl group was removed by treatment with 70 ml NH_3/MeOH for 5 hours at room temperature. Evaporation left an oil which was purified on silica gel (CH_2Cl_2 - MeOH, 98:2) to a yield of 400 mg of the debenzoylated derivative as an oil.

The obtained oil was treated with 50 ml 80% acetic acid at 80°C for 5 hours. After evaporation and co-evaporation with toluene the residue was dissolved in water and washed with diethylether. The water layer was evaporated and the precipitate crystallized out of MeOH-Et₂O with a yield of 234 mg of the compound 20 (0.97 mmol, 80%).

UV (MeOH) : λ_{max} 276 nm (8200)

CIMS (C_4H_{10}) m/e : 242 (MH^+)

¹H NMR (DMSO-d₆) δ 1.47-1.87 (m, H-3), 1.91-2.28 (m, H-3'), 2.95-3.30 (m, 1H, H-2), 3.35-4.10 (m, 5H), 4.52 (m, 2H, 6'-OH + H-5'), 4.85 (d, J=4.8Hz, 4'-OH), 5.66 (d, J=7.5Hz, H-5), 6.99 (s, NH₂), 7.97 (d, J=7.5Hz, H-6) ppm.

¹³C NMR (DMSO-d₆) δ 35.7 (C-3'), 51.5 (C-2'), 61.0, 61.2 (C-4', C-6'), 67.9 (C-1'), 82.9 (C-5'), 93.7 (C-5), 144.3 (C-6), 156.3 (C-2), 165.7 (C-4) ppm.

Anal. ($\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_4$)

Calculated: C, 49.79; H, 6.27; N, 17.42

Found: C, 49.85; H, 6.27; N, 17.20

Anti-viral tests

The anti-viral activity of the compounds according to the invention in respect of the herpes virus and related viruses is illustrated by the following tests. In these 5 tests the effect was determined of the 1,5-anhydrohexitol nucleoside analogues according to the invention on the growth and yield of the virus in cell cultures.

The compounds used are described in the examples together with a number of well known anti-herpes agents from 10 the prior art, that is, BVDU or E-5-(2-bromovinyl)-2'-deoxyuridine, Ribavirin or 1-ribofuranosyl-3-carbamoyl-1,2,4-triazol, (S)DHPA or (S)-9-(2,3-dihydroxypropyl)-adenine and C-c³ Ado or carbocyl 3-deaza adenosine.

The compounds were tested against herpes simplex virus 15 type 1 (HSV-1), herpes simplex virus 2 (HSV-2) and vaccinia virus (VV). These viruses were cultured in human embryonal skin muscle (E₆SM) fibroblast cell cultures.

The tests were based on the inhibition of virus-induced cytopathogenesis in cell cultures. A standard procedure is 20 described by De Clercq et al., J. Infect. Dis. 141, 463 (1980) which is incorporated herein by way of reference.

Test 1

In this test the inhibiting activity of the test 25 compounds against viruses was measured in E₆SM cell cultures. The cells were cultured to confluence in microtitre (R) plates and then inoculated with 100 CCID₅₀ virus, wherein a CCID₅₀ of the virus corresponds with the virus dose required to infect 50% of the cell cultures. After a 30 virus adsorption period of an hour remaining virus was removed and the cell cultures incubated in the presence of different concentrations of the test compounds (varying from 0.001 µg/ml to 400 µg/ml). For each virus cell system the ED₅₀ was determined, that is, the concentration of the 35 compound required to suppress the cytopathic effect of the virus by 50%. This cytopathic effect was noted as soon as it reached completion in the non-treated, virus-infected cell cultures. In addition the minimum cytotoxic concentration of

each compound was measured. The results are shown in table I.

Test 2

Further, the inhibiting effect of the test compounds on virus multiplication in E₆SM cell cultures was measured making use of herpes simplex viruses missing a specific thymidine kinase. Three different strains were used: TK⁻ Cheng, TK⁻ Field and a clinically isolated strain VMW/837. The results are shown in table II.

10

Table I

Cytotoxicity and anti-viral activity of nucleoside analogues in human embryonal skin muscle (E₆SM) fibroblast cultures.

15	Compound	Minimum cytotoxic concentration ^a (μg/ml)	Minimum inhibiting concentration ^b ED ₅₀ (μg/ml)		
			HSV-1 (KOS)	HSV-2 (G)	VV
20	10	>400	7	7	20
	13	>400	0.2	0.1	2
	18	>400	0.07	0.07	150
	17	>400	40	150	>200
	25 20	>400	0.7	0.04	0.7
30	IDU	>400	0.2	0.2	0.2
	BVDU	>400	0.004	10	0.2
	(S)-DHPA	>400	70	300	20
	Ribavirin	>400	70	70	70
	C-c ³ Ado	>400	>400	40	0.7

^aRequired to cause a microscopically detectable change in the normal cell morphology

^bRequired to reduce the virus-induced cytopathogenesis by 50%

Table II

Cytotoxicity and anti-viral activity of nucleoside analogues in human embryonal skin muscle (E₆SM) fibroblast cultures.

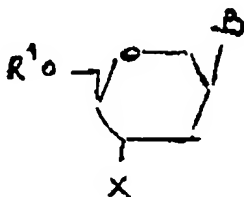
5	Compound	Minimum cytotoxic concentration ^a (μg/ml)	Minimum inhibiting concentration ^b ED ₅₀ (μg/ml)		
			HSV-1 TK ⁻ Cheng C 158/77	HSV-2 TK ⁻ Field C 137/101	VV VMW/837 #3
10	10	>400	150	70	20
	13	>400	20	20	2
15	18	>400	>200	>200	1
	17	>400	>200	>200	>200
	20	>400	2	2	2
<hr/>					
	IDU	>400	10	10	7
20	BVDU	>400	10	10	4
	(S)-DHPA	>400	400	>400	>400
	Ribavirin	>400	>400	>400	>400
	C-c ³ Ado	>400	70	>400	>400

25 ^aRequired to cause a microscopically detectable change in normal cell morphology

^bRequired to reduce virus-induced cytopathogenesis by 50%

CLAIMS

1. 1,5-anhydrohexitol nucleoside analogues represented by the general formula I:



(I)

wherein:

5 B is a heterocyclic ring which is derived from the group which consists of pyrimidine and purine bases, and X represents hydrogen, azido, F, Cl, Br, I, amino, -NHR², -N(R²)₂, -OR², -SR² or CN;

10 wherein R¹ and R² are the same or different and hydrogen, alkyl, acyl or phosphate moieties are represented; wherein:

15 - alkyl is a saturated or unsaturated, substituted or non-substituted hydrocarbon radical with 1-20 carbon atoms and straight or branched chain, and
 - acyl is an alkanoyl or aroyl moiety, wherein alkanoyl is an alkylcarbonyl radical, wherein alkyl is as described above and aroyl represents benzoyl, substituted benzoyl or naphthoyl;

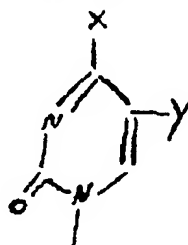
or

20 X represents hydrogen and a double bond is situated between the 3- and 4- position of the 1,5-anhydrohexitol ring, in addition to pharmaceutical salts and esters thereof.

25 2. 1,5-anhydrohexitol nucleoside analogues as claimed in claim 1, characterized in that the hexitol has the D-configuration and the base moiety and the X substituent both have the (S)-configuration.

3. 1,5-anhydrohexitol nucleoside analogues as claimed in claim 1, characterized in that X represents hydroxyl in the (S)-configuration.

4. 1,5-anhydrohexitol nucleoside analogues as claimed in claim 1, characterized in that the heterocyclic ring derived from the group consisting of pyrimidine and purine bases is represented by the formula III:



(III)

wherein:

10 X represents OH, NH₂, NHQ,
wherein:

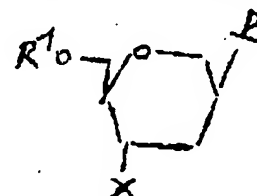
Q represents OH or C₁₋₅ alkyl;

15 Y represents H, F, Cl, Br, C₁₋₅ alkyl, haloethyl or CH=CH-R, wherein R represents hydrogen, halogen or C₁₋₅ alkyl and wherein haloethyl contains 1-4 F, Cl or Br atoms.

5. 1,5-anhydrohexitol nucleoside analogues as claimed in claim 1, characterized in that the heterocyclic ring derived from the group consisting of pyrimidine and purine bases is chosen from the group which consists of substituted and non-substituted adenine, guanine, hypoxanthine and xanthine.

6. 1,5-anhydrohexitol nucleoside analogues as claimed in claim 1, characterized in that aza-, deaza-, deoxy- or deamino- analogues of each of the heterocyclic rings, which if desired carry one or more substituents as defined in any of the foregoing claims, are present in the compounds of formula I.

7. Method for preparing 1,5-anhydrohexitol analogues represented by the general formula I



wherein:

B is a heterocyclic ring which is derived from the group which consists of pyrimidine and purine bases, and

X represents hydrogen, azido, F, Cl, Br, I, amino, $-NHR^2$, $-N(R^2)_2$, $-OR^2$, $-SR^2$ or CN;

wherein R^1 and R^2 are the same or different and hydrogen, alkyl, acyl or phosphate moieties are represented; wherein:

- alkyl is a saturated or unsaturated, substituted or non-substituted hydrocarbon radical with 1-20 carbon atoms and straight or branched chain, and

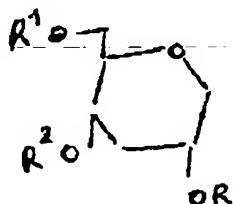
- acyl is an alkanoyl or aroyl moiety, wherein alkanoyl is an alkylcarbonyl radical, wherein alkyl is as described above and aroyl represents benzoyl, substituted benzoyl or naphthoyl;

or

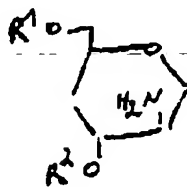
X represents hydrogen and a double bond is situated between the 3- and 4- position of the 1,5-anhydrohexitol ring, in addition to pharmaceutical salts and esters thereof, which method comprises the steps of:

a) first manufacturing suitably protected 1,5-anhydrohexitol derivatives represented by the general

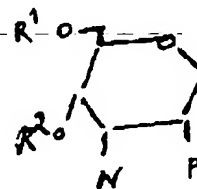
formulas X, XI and XIII



X



XI



XIII

wherein R^1 and R^2 represent protective groups (for example $R_1, R_2 = C_6H_5-CH=$) and R represents a leaving function (for example $R=SO_2CH_3, SO_2C_6H_4CH_3, SO_2C_6H_4Br$) or $R=H$;

5 b) making use of the derivatives X for alkylizing a heterocyclic ring which is derived from the group of pyrimidine and purine bases;

c) making use of the derivatives XI for constructing a heterocyclic ring from the amine; and

10 d) using the derivative XIII, wherein P represents -OR, wherein R represents a leaving function as stated above or P and N are components of an epoxidization for introducing the heterocyclic ring in the 2-position followed by removal of the hydroxyl group in the 3-position;

15 e) if necessary converting the obtained compound to pharmaceutically acceptable salts or esters thereof.

8. Pharmaceutical composition with anti-viral activity against herpes viruses, pox viruses and related viruses, which composition comprises as an active ingredient a 1,5-anhydrohexitol nucleoside analogue of formula I, 20 wherein:

B is a heterocyclic ring which is derived from the group which consists of pyrimidine and purine bases, and

X represents hydrogen, azido, F, Cl, Br, I, amino, $-NHR^2$, $-N(R^2)_2$, $-OR^2$, $-SR^2$ or CN;

25 wherein R^1 and R^2 are the same or different and hydrogen, alkyl, acyl or phosphate moieties are represented; wherein:

30 - alkyl is a saturated or unsaturated, substituted or non-substituted hydrocarbon radical with 1-20 carbon atoms and straight or branched chain, and
- acyl is an alkanoyl or aroyl moiety, wherein alkanoyl is an alkylcarbonyl radical, wherein alkyl is as described above and aroyl represents benzoyl, substituted benzoyl or naphthoyl;

35 or

X represents hydrogen and a double bond is situated between the 3- and 4- position of the 1,5-anhydro-hexitol ring.

9. Pharmaceutical composition as claimed in claim 8,
5 characterized by anti-viral activity against herpes-like viruses, which are chosen from the group which consists of herpes simplex virus type I (HSV-1), herpes simplex virus type 2 (HSV-2), Varicella zoster virus (VZV) and cytomegalo virus (CMV) as well as against pox viruses, for instance
10 vaccinia virus (VV).

10. Pharmaceutical composition as claimed in claim 8, characterized in that the composition contains the active ingredient in a concentration between about 0.1 and 100% by weight.

15 11. Pharmaceutical composition as claimed in claim 9, characterized in that the composition takes the form chosen from the group consisting of powders, suspensions, solutions, sprays, emulsions, salves and creams.

20 12. Use of the 1,5-anhydrohexitol nucleoside analogues of formula I as defined in claim 1 as an agent with biological activity.

25 13. Use of 1,5-anhydrohexitol nucleoside analogues of formula I as defined in claim 1 as an agent with anti-viral activity against herpes viruses, pox viruses and related viruses.

14. Use of 1,5-anhydrohexitol nucleoside analogues of formula I as defined in claim 1 for the preparation of a pharmaceutical composition with anti-viral activity against herpes viruses, pox viruses and related viruses.

30 15. Method for treating virus diseases caused by herpes viruses, pox viruses and related viruses, which consists of a 1,5-anhydrohexitol nucleoside analogue of formula I being administered to a patient suffering from such a disease, wherein

35 B is a heterocyclic ring which is derived from the group which consists of pyrimidine and purine bases, and X represents hydrogen, azido, F, Cl, Br, I, amino, $-NHR^2$, $-N(R^2)_2$, $-OR^2$, $-SR^2$ or CN;

wherein R¹ and R² are the same or different and hydrogen, alkyl, acyl or phosphate moieties are represented; wherein:

5

- alkyl is a saturated or unsaturated, substituted or non-substituted hydrocarbon radical with 1-20 carbon atoms and straight or branched chain, and

10

- acyl is an alkanoyl or aroyl moiety, wherein alkanoyl is an alkylcarbonyl radical, wherein alkyl is as described above and aroyl represents benzoyl, substituted benzoyl or naphthoyl;

or

15

X represents hydrogen and a double bond is situated between the 3- and 4- position of the 1,5-anhydro-hexitol ring, or a pharmaceutically acceptable salt or ester thereof.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/BE 93/00036

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C07H19/04; C07D473/04; C07D405/04; A61K31/70		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C07H ; C07D ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	EP,A,0 217 580 (THE WELLCOME FOUNDATION) 8 April 1987 see the whole document ---	1-15
Y	EP,A,0 409 227 (AKADEMIE DER WISSENSCHAFTEN DER DDR) 23 January 1991 see page 2, line 1 - page 9, line 38 ---	1-15
Y	WO,A,9 001 036 (MEDIVIR AB) 8 February 1990 see abstract --- -/--	1-15
<p>¹⁰ Special categories of cited documents :¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
09 SEPTEMBER 1993	28.09.93	
International Searching Authority	Signature of Authorized Officer	
EUR PEAN PATENT OFFICE	SCOTT J.R.	

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	JOURNAL OF MEDICINAL CHEMISTRY vol. 30, no. 6, June 1987, WASHINGTON US pages 1044 - 1054 L.D.NORD ET AL. 'Synthesis, Structure, and Biological Activity of Certain 2-Deoxy-B-D-ribo-hexopyranosyl Nucleosides and Nucleotides.' cited in the application see the whole document ---	1-15
Y	JUSTUS LIEBIGS ANNALEN DER CHEMIE vol. 1990, no. 11, November 1990, WEINHEIM DE pages 1079 - 1082 P.HANSEN ET AL. 'Synthesis of 3'-Azido-2', 3'-Dideoxy-B-D-arabino-hexopyranosyl Nucleosides.' cited in the application see the whole document -----	1-15

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

BE 9300036
SA 75964

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

09/09/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0217580	08-04-87	AU-B- 595832	12-04-90
		AU-A- 6270286	19-03-87
		CA-A- 1302263	02-06-92
		GB-A- 2181128	15-04-87
		AT-B- 390000	26-02-90
		AT-B- 392794	10-06-91
		AU-B- 572019	28-04-88
		CA-A- 1238277	21-06-88
		DE-A- 3608606	18-09-86
		DE-A- 3645058	02-02-89
		DE-A- 3645059	05-01-89
		DE-A- 3687069	10-12-92
		EP-A,B 0196185	01-10-86
		EP-A,B 0291633	23-11-88
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